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Neonatal Chiordecone Exposure Alters Behavioral Sex Differentiation in Female Hamsters

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16. ABSTRACT

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Neonatal Chlordecone Exposure Alters Behavioral Sex Differentiation In Female Hamsters

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ABSTRACT: *The present study was designed in order to determine if exposure to the weakly estrogenic pesticide Chlordecone during a critical period of behavioral sex differentiation of the brain could masculinize and defeminize the behavior of female hamsters. In the first experiment female hamsters were given a single s.c. injection of either Chlordecone (Kepone) at 1.0 mg/pup or the vehicle at two days of postnatal age while in the second experiment, the pups were injected with either Chlordecone at dosages of 0.25, 0.50 or 1.00 mg/pup or estradiol benzoate (EB) at 20 or 100 micrograms/pup at four days of age in order to compare the long-term effects of the two estrogenic substances on sexually dimorphic behaviors. In both experiments behavioral estrous cyclicity was measured, then females were ovariectomized, treated with the male hormone testosterone propionate and tested for their ability to mount a sexually receptive female. Mounting behavior is not normally displayed by female hamsters that have not been masculinized by neonatal treatment with testosteone or estradiol. In addition, open field ambulation and rearing behaviors were measured in the intact males and females in the second experiment. These behaviors are also sexually dimorphic as a result of the organizing influence of androgens and estrogens on the brain of the neonatal hamster.*

Females exposed as neonates to the high dose of (100 μ-mg) EB were both defeminized and masculinized by this treatment, whereas the females treated with the lower dose of EB (20 μ-mg) or with Chlordecone (.5 and 1.0) mg were masculinized but not defeminized, and effect consistent with exposure to low doses of testosterone. The open field ambulation and rearing behavior levels also were altered by both neonatal estradiol and Chlordecone treatment. The treated females behaved like males in that they ambulated and reared less than did the control females; activity levels of the males were unaffected. These data demonstrate that Chlordecone acts like a weak estrogen in the brain of the neonatal hamster, altering the process of behavioral sex differentiation such that females are masculinized and show abnormal bisexual behavior.

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INTRODUCTION

Chlordecone (Kepone, 1, 1a, 3, 3a, 4, 5, 5a, 5b, 6-decachlorooctahydro-1, 3, 4-metheno-2H-cyclobuta (cd)entalen-2-one) is a chlorinated hydrocarbon insecticide which, until recently, was produced in the United States. Chlordecone produces signs of neurotoxicity, hepatic dysfunction, and reproductive disorders in man. Chemical workers employed in its manufacturing in Hopewell, Virginia reported tremors, headaches, mental and visual disturbances, and sterility as judged by oligospermia and hypomotile sperm (Cannon *et al.*, 1978). Dietary exposure at highly toxic dosage levels in rats (50 and 80 ppm) causes testicular atrophy at three months and death at six months (Larson *et al.*, 1979). Administration of less toxic doses causes a cessation of breeding in the treated females, but not in treated males (Huber, 1965; Cannon and Kimbrough, 1979). In these studies fertility was still inhibited for the first few weeks following the termination of treatment but reproductive ability resumed following a recovery period. Estrogens are very potent hormones and chronic dietary exposure to estrogenic substances is toxic to both male and female reproductive systems (Shardein, 1980; Heywood and Wadsworth, 1980). It is likely that many, if not all, of the reproductive effects of Chlordecone are due to this hormonal activity. Previous examination of the toxicology of Chlordecone demonstrated that it is estrogenic *in vivo* and *in vitro* (Eroschenko, 1981); dietary administration of Chlordecone produces constant vaginal cornification and reduction in fertility (Good *et al.*, 1965; Huber, 1965); Chlordecone is uterotrophic in immature rats (Gellert, 1978) and effects the development of the uterus, oviduct and vagina of the neonatal mouse (Eroschenko, 1982; Eroschenko and Mousa, 1979); it competes with estradiol for binding to the cytoplasmic receptor *in vitro* and *vivo* and induces nuclear accumulation of estrogen receptor sites *in vitro* and it stimulates the synthesis of the progesterone receptor *in vivo*, an estrogen receptor-mediated process (Hammond *et al.*, 1979).

Gellert (1978) administered Chlordecone by s.c. injection during the critical period of behavioral sex differentiation and found that the females were partially defeminized and developed persistent vaginal estrus (PVE) at six months of age. This syndrome, the delayed anestrus syndrome (DAS) is characteristic of neonatally androgen and estrogen treated female rats (Gorski, 1971). Some of the reproductive effects which result from the neonatal hormone treatments in females or occur normally in the male at this time are related to structural modifications of the medial preoptic area of the rat brain, which is larger in the male (Gorski, *et al.*, 1978). This effect differs from the other reported effects of Chlordecone in that defeminization and masculinization are irreversible alterations of reproductive development.

Unlike rats, some other mammalian species, including primates, are not defeminized by hormonal treatment during this critical developmental period, and the extrapolations concerning defeminization effects to man has been questioned (Baum, 1979). These species are rather more easily masculinized in their behavioral patterns as a result of neonatal hormonal treatment, but they are not defeminized and continue to cycle and breed normally. The hamster is an attractive species for studying the effects of chemicals on behavioral sex differentiation because females can be masculinized more easily than defeminized. Neonatal treatment of the female hamster with 3 micrograms of testosterone propionate, masculinized the treated females enabling them to display male-like mounting behavior after being treated with testosterone as adults, but defeminization occurred only at doses more than two orders of magni-

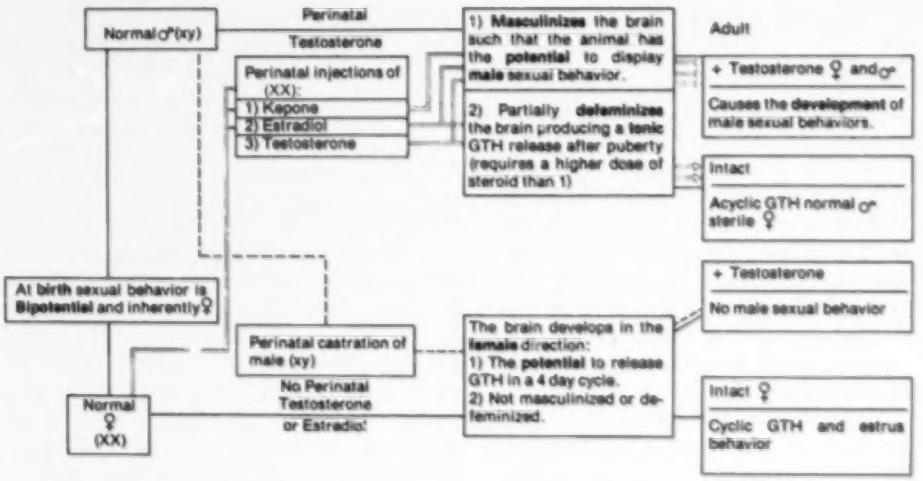
Sexual Differentiation of the Hamster
Normal ♂(xy)
Perinatal
Testosterone
Perinatal injections of (XX):
1) Kepone
2) Estradiol
3) Testosterone
1) Masculinizes the brain such that the animal has the potential to display male sexual behavior.
2) Partially defeminizes the brain producing a tonic GTH release after puberty (requires a higher dose of steroid than 1)
Adult
+ Testosterone ♀ and ♂
Causes the development of male sexual behaviors.
Intact
Acyclic GTH normal ♂ sterile ♀
At birth sexual behavior is Bipotential and inherently ♀
Perinatal castration of male (xy)
No Perinatal Testosterone or Estradiol
Normal ♀ (XX)
The brain develops in the female direction:
1) The potential to release GTH in a 4 day cycle.
2) Not masculinized or defeminized.
+ Testosterone
No male sexual behavior
Intact ♀
Cyclic GTH and estrus behavior

FIG. 1 Behavioral sex differentiation of the central nervous system occurs prenatally in humans but perinatally in the hamster. The undifferentiated animal develops into a male due to testicular testosterone (T), or a female if T is not present.

tude above this masculinizing dose (Whitsett and Vandenbergh, 1975). The neonatal hormonal treatment organized the brain in a male-like direction and adult testosterone administration activates the behavior. In the absence of the early organizing influence of the hormones the females do not respond to the activational testosterone treatment (Fig.1).

The present investigation was designed to determine if Chlordecone administration to the female hamster during a critical period of behavioral sex differentiation would produce irreversible behavioral alterations by masculinizing the brain. In addition to those behaviors associated with copulation, many other nonreproductive behaviors are also sexually dimorphic (Table 1). A sex difference in open field activity in the hamster occurs as a consequence of the neonatal hormone milieu, with males and masculinized females being less active (Swanson, 1966, 1967). Consequently, we also measured the open field activity of male and female hamsters exposed to estradiol or Chlordecone.

MATERIALS AND METHODS

General Methods

Timed-pregnant Golden Syrian hamsters were obtained from Engle Laboratories. Day 0 of pregnancy was recorded upon successful copulation. Pregnant animals were individually housed in cages (23 x 15 x 45 cm) on pine shavings and maintained under conditions of constant temperature (22-24 degrees C) with controlled lighting (14 h light/10 h dark, lights out at 1200 EST). They were fed

Wayne Lab Blox (Allied Mills) and tap water *ad lib*. Technical grade Chlordecone was obtained from Allied Chemical Co. (lot no. SN 18 104-4) (courtesy of Ralph Linder of our laboratory).

Methods of Experiment 1

In the first experiment Chlordecone was given by s.c. injection in 20 microliters of DMSO (Dimethyl Sulfoxide, Analytical Reagent Grade). The pups in eight litters were injected with the vehicle on day 2 (day of birth is day 1) (injected controls) of postnatal age, eight litters were given 1 mg/pup of Chlordecone and the third group of four litters was undisturbed (uninjected controls). DMSO was used as the vehicle because it is an excellent solvent, allowing us to dissolve 1 mg of chemical in 20 μl, a concentration which cannot be achieved using corn oil. This small volume is used to avoid leakage after injection. In addition, studies in our laboratory using hamsters and rats indicate that this volume of DMSO is without affect on growth, survival or reproductive development (Gray, unpublished). Pups were checked daily following treatment and the percentage of female pups with patent vaginas was determined at nine and 15 days of age. The females were weaned at 23 days of age and housed with littermates. The vaginal and behavioral estrous cyclicity was measured over one four day cycle from 43 to 46 days of age. Vaginal cyclicity was determined using the method of Orsini (1961) and behavioral estrus was determined by placing the female in the home cage of a proven stud male and observing if she displayed the posture of lordosis.

At 70 days of age the females were ovariectomized and a 25 mg pellet of testosterone propionate was implanted beneath the skin. Thirty days later these females were tested for their ability to mount a sexually receptive female hamster. At the onset of the dark period each experimental animal was placed in the cage with a sexually receptive female and the number of rear mounts with pelvic thrusts by the experimental female directed towards the stimulus female was counted. The experimental female was given 300 s to mount the stimulus female or the test was terminated. The number of mounts that occurred in a three minute period following the first mount were counted.

Methods of Experiment 2

In the second experiment Chlordecone or estradiol benzoate were administered by s.c. injection at four days of age. Thirty-nine litters were used in the present experiment with six or seven litters in each of the six dosage groups. The control group received a single injection of 20 microliters of the solvent DMSO. Chlordecone was administered at .25, .5 or 1.0 mg/pup and estradiol benzoate at 20 or 100 micrograms per pup. The females and males were weaned at 30 days and the pups were housed in unisexual groups with littermates.

The flank gland of the males, an androgen dependent tissue, was measured throughout puberty (Vanderbergh, 1973). Open field activity, a sexually dimorphic behavior, was measured in males and females from the six treatment groups starting at 110 days of age in a circular open field (72 cm in diameter) (Kavlock *et al.*, 1979; Gray *et al.*, 1981). A five minute observation of the open field behaviors was conducted in the dark phase of the animals activity cycle. An animal was placed in the central portion of the circular open field, and the latency to enter a peripheral "square", the number of "squares" entered, and the number of rears were measured. Behavioral estrous cyclicity was measured starting at 170 days over two cycles, after which the females were ovariectomized at 180 days. One week later the females were given a series of five injections of 0.1 mg of testosterone propionate (in corn oil) every

other day over a ten day period. On the day of the last injection the experimental females were paired with a sexually receptive female, and the number of mounts were counted. At this time the mounting behavior of a few males from the untreated group and the high dosage groups was measured and following behavioral testing the males were necropsied and the testes, and cauda epididymides were weighed. The measurement of cyclicity and mounting behaviors was delayed in this experiment as compared with the first for two reasons: 1) the onset of acyclicity in defeminized female rats is delayed at low doses (DAS syndrome) and testing the females at an older age increases the likelihood of detecting an effect (Gellert, 1978); and 2) an increase in mounting behavior by the treated females at this age, in conjunction with the results of experiment 1 in the younger Chlordecone treated females, would demonstrate that this effect is present at 80 days of age and persists until the females are 210 days old.

Experimental Design

In the present experiments we elected to dose all the pups within a litter with the same dose, treatment being a "between litter" effect, rather than using a "within litter" design in which each of the pups in a litter would receive a different treatment. This decision was based on previous analyses which indiciated that litter effects on the behaviors measured in the present study are undetectable and concern that the control pups might be exposed inadvertently to Chlordecone in the milk. Lactating dams routinely ingest the pups urine and fecal material in order to keep the nest area clean and cannibalize dead or moribund pups, including those dying as a result of treatment. Thus, these behaviors result in significant maternal exposure as indicated by the observation of Chlordecone-induced tremors in those dams in the 1 mg dosage group that cannibalized their pups. Therefore, although a within-litter design is statistically preferable, because litter effects are not confounded with treatment effects., it would be unacceptable for the present study and for many developmental toxicology studies. However the dosing of animals in litters raises a question concerning the choice of an experimental unit for statistical analysis. This question was answered empirically by comparing the between- and within-litter variances in behavioral scores. An *F* test was used to determine the significance of the two components. If individuals were behaving independently of others in their litters, the ratio of between-litter to within-litter variances should equal one and the individual would be acceptable as the experimental unit. A statistically significant *F* ratio would indicate that the performance of an individual hamster was similar to its littermates. In such a situation, the litter should be regarded as the proper experimental unit. A similar rationale has been used in evaluating litter effects upon open-field activity (Denenbert *et al.*, 1978) and aggressive behavior (Gray *et al.*, 1978). Obtained *F* ratios of approximately one indicated the acceptability of using the individual hamster, rather than the litter containing several animals, as the unit of analysis in the experiments of the present study.

Statistical Methods

The data from the first experiment were analyzed using the General Linear Models (GLM) procedure available on the 1979 edition of the Statistical Analysis System (SAS) (Helwig and Council, 1979). Statistical significance was determined using the partial sums of squares because sample sizes were unequal. In the second experiment the data from the males was analyzed separately from the females because our *a priori* hypothesis was that the behavior of the females would become male-like but that the males would be unaffected. To ease interpretation of the significant effects noted in the females behavior, the estradiol and Chlordecone dose response data were also analyzed separately. A discriminant function analysis of the open field activity in untreated males and females including the ambulation, rearing and latency

TABLE 1. Sexually Dimorphic Behaviors of the Hamster.

These are behaviors where a) the potential to display a posture does not exist in one of the sexes or b), more typically, the behavior occurs at different rates or intensities in the two sexes. Differences between the sexes exist in these behaviors as a consequence of the perinatal and adult hormonal milieu.

1. Aggressive rough and tumble play - Female < Male
2. Open-field behaviors including
   a. Ambulation - Female > Male
   b. Rearing - Female > Male
   c. Scrabbling - Female > Male
   d. Latency to ambulate - Male > Female
3. Food hoarding - Female > Male
4. Nest building - Female > Male
5. Maternal behaviors - Female > Male
6. Gnawing - Female > Male

behaviors, was calculated to derive a set of weighting factors that optimized the difference between the sexes. With these weighting factors (a1, a2, a3) a new composite variable (L) for each treated and control animal can be calculated from the equation L = a1(ambulation) + a2(rearing) +a3(latency) +a4. This single new variable is a linear weighted sum of the three open field behaviors and a positive score indicates that an animal behaved like a control female in the open field while a negative value indicates that the animal behaved like a male. We expected that neonatal estrogen or Chlordecone exposure would cause the discriminant function scores (L) of treated females to become more malelike, shifting towards zero or becoming negative. A *t*-test of the significance of the treatment effects on the L values is identical to the results one would obtain from a multivariate analysis of variance (Li, 1964).

RESULTS

The results of the first experiment are shown in Table 2. The percentage of females with patent vaginas per litter was significantly accelerated at nine days of age by Chlordecone treatment on day two ($p < .005$); these females mounted the stimulus female significantly more than the untreated females ($p < .001$) when tested as adults. Although these females were masculinized by Chlordecone, they were not defeminized as all the females had normal estrous cycles. Chlordecone treatment on day two did not cause tremors as it does in adult animals, but there was significant mortality (45%) in this treatment group by day nine as compared with controls (1%).

DMSO injection did not affect the growth, viability or reproductive development as measured in the present experiment (Table 2) as compared with that of

TABLE 2. The Effect of Neonatal Exposure to Chlordecone at 1 mg/pup at 2 Days of Age on the Mounting Behavior of Young Adult Female Hamsters Following Ovariectomy and Testosterone Treatment. (An increase in this male-typical behavior over the control value indicates that the brain of the treated female has been masculinized by Chlordecone administration on day 2. However the vaginal and behavioral estrous cyclicity of the treated females was normal demonstrating that they not been defeminized).

Variable	Control (vehicle)	Chlordecone on day 2 1 mg/pup	Control (uninjected)
Number of Head and Side Mounts	.67 (.38) 14[a]	2.60 (.97) 5[a]	
Number of Rear Mounts	.26 (.20) 14[a]	4.60 (1.60) 5[a]**	
Total Mounts	.93 (.54) 14[a]	7.2 (2.1) 5[a]**	
Percentage of Females with Normal Vaginal and Behavioral Cyclicity	100% (22/22)	100% (8/8)	100% 16/16)
Percent Survival to Day 9	99%	61%	94%
Female Weight on Day 9 (g)	9.9 (.47) 8	11.2 (.50) 5	10.1 (.54) 4
Percentage of Females with Patent Vaginas			
On Day 9	45% (13) 8[b]	100% (00) 5[a]**	31% (14) 4
On Day 15	87% (09) 8[b]	95% (12) 5	77% (14) 4

[a]Values are means (standard errors) numbers of females.
[b]Values are litter means (standard errors) numbers of litters.
*$p < .01$ Different from control by a *t*-test.
**$p < .05$ Different from control by a *t*-test

the uninjected controls. These results are consistent with our earlier and more extensive observations on the toxicity of a single 20 μl injection at this age. The analysis for a litter effect on the mounting behavior of the ovariectomized testosterone treated females indicated that litter mates behaved independently of one another with $F(10,11) = 1.4$, $p > .28$.

In the second experiment the open field behaviors and the sexual behavior of the treated females were masculinized (Table 3 and 4). Females treated with estradiol benzoate and Chlordecone had longer latencies to begin ambulating, and they ambulated and reared less than the control females. Thus their open field behavior was somewhat male-like. The discriminate function analysis of the control male and female open field behaviors yielded an equation for the new variable of L = 0.13266(ambulation) + 0.24439 (rears) - .51188(latency) -25.0307. Using this criterion to classify the animals behavior as male or female-like indicated that although there was no overlap between untreated males and females (all the females had positive scores and the

TABLE 3. The Effects of a Single S.C. Injection of Estradiol Benzoate or Chlordecone at Four Days of Age on Sexually Dimorphic Open Field Behaviors in the Adult Hamster

		Treatment					
		Control	Chlordecone			Estradiol	
Open Field Behavior	Variables	0	250 μg	500 μg	1000 μg	20 μg	100 μg
Females	% with (—) L	0%	8%	33%	44%	18%	29%
	Ambulation	174 (4.8)[a]	181 (7.5)	167 (7.0)	155 (3.8)*	177 (4.8)	160 (4.9)*
	Rearing	43 (2.2)	37 (2.7)	29 (5.3)**	28 (1.7)**	34 (2.7)**	37 (2.1)
	Latency	1.9 (0.3)	3.8 (0.6)**	3.1 (0.5)	4.6 (0.9)**	3.5 (0.6)	5.8 (1.2)**
	Discriminant Function (L)	+7.6 (0.9)	+6.2 (1.4)	+2.7 (1.9)*	+.03 (1.2)**	+4.9 (1.2)	+3.3 (1.3)*
	No. Tested	17	13	9	9	17	17
Males	Ambulation	122 (5.5)	128 (7.2)	135 (10.0)	141 (13.4)	121 (7.5)	125 (12.8)
	Rearing	24 (2.6)	23 (2.9)	21 (3.3)	24 (2.2)	22 (1.7)	26 (2.6)
	Latency	5.8 (1.5)	7.8 (1.2)	8.4 (2.2)	8.8 (2.0)	16.4 (3.1)*	14.1 (3.8)*
	Discriminant Function (L)	—6.0 (1.3)	—6.3 (2.0)	—5.4 (2.3)	—4.9 (2.6)	—12.0 (1.8)*	—9.4 (3.5)
	No. Tested	8	8	8	8	8	8

[a]Values are X (SE)

*$p < .05$ Different from control value

**$p < .01$ Different from control value

TABLE 4. The Effects of a Single S.C. Injection of Estradiol Benzoate or Chlordecone at Four Days of Age on Sexually Dimorphic Reproductive Behaviors in the Adult Female Hamster.

		Treatment					
		Control	Chlordecone			Estradiol	
	Variables	0	250 μg	500 μg	1000 μg	20 μg	100 μg
Test of Mounting Behavior	No. of Mounts	0.0 (0)	0.7 (0.7)	4.8 (1.6)*	1.5 (0.9)	4.3 (2.0)	6.8 (2.2)**
	Percent Mounting	0%	8%	83%	37%	50%	73%
	Percent Fighting During Test	45%	42%	17%	13%	0%	0%
	No. Tested	11	12	6	8	6	11
Behavioral Estrous Cyclicity	Percent of Females with Abnormal Cycles	4%	18%	20%	21%	7%	75%
	No. Tested	26	17	10	14	14	20

* $p < 0.5$ Different from control value

**$p < .01$ Different from control value

TABLE 5. The Effects of Neonatal Estrogen or Chlordecone Administration on the Sexual Development of the Male Hamster.

	Treatment					
	Control	Chlordecone			Estrogen	
Variable		0.25mg	0.50mg	1.0mg	20μg	100μg
FGD 34 days[a]	2.7 ± 0.1[b]	2.9 ± 0.2	2.8 ± 0.2	2.6 ± 0.2	2.6 ± 0.2	2.7 ± 0.3
FGD at 39 days	3.1 ± 0.2	3.3 ± 0.2	3.0 ± 2.8	2.9 ± 0.2	2.9 ± 0.1	3.0 ± 0.3
FGD at 48 days	3.4 ± 0.2	3.7 ± 0.2	3.4 ± 0.2	3.2 ± 0.3	3.3 ± 0.1	3.5 ± 0. 2
FGD at 58 days	4.8 ± 0.3	4.8 ± 0.2	4.4 ± 0.2	4.4 ± 0.1	4.3 ± 0.1	4.7 ± 0.3
Male sex behavior, number of mounts	11.6 ± 2.9	—	—	11.6 ± 2.4	—	11.8 ± 1.8
Paired testes weight (g)	4.23 ± 0.17	—	—	3.39 ± 0.20*	—	3.30 ± 18*
Epididymal weight (g)	0.997 ± .030	—	—	0.660* ± .086	—	50% cystic

[a]FGD: flank gland diameter in millimeters, a bioassay for testosterone

[b]Values are X ± SE

*$p < .05$ different from control mean

males all had negative scores), many of the treated females had negative L values and were classified as males (Table 3).

In the second experiment estradiol benzoate treatment defeminized and masculinized female sexual behavior (Table 4). Chlordecone treatment on day four masculinized the females so that they mounted a sexually receptive female, but they were not defeminized. The sexual behavior of treated males was normal although testis and other reproductive organ weights were reduced at necropsy (Table 5). Chlordecone treatment resulted in significantly increased mortality prior to weaning in the 1 mg (70%) dosage group but not at 0.5 mg (35% mortality) or 0.25 mg (4% mortality) as compared to controls (13%). Estrogen did not cause mortality among the pups with 0% and 5% dying in the 100 and 200 microgram groups respectively.

Results of the analyses for litter effects on open-field and mounting behaviors in the second experiment indicated in all cases that littermates behaved independently of one another and that the individual rather than the litter was the appropriate unit of analysis. The *F* values for ambulation, rearing, latency to ambulate and mounting behaviors are: $F(30,88) = 1.1$, $p > .25$; $F(30,88) = 1.07$, $p > .4$; $F(30,88) = 0.95$, $p > .5$; and $F(21,53) = 0.9$, $p > .6$ respectively.

DISCUSSION

Results of the present study demonstrate that a brief exposure of hamsters to the hormonally active chemical Chlordecone during a sensitive development period (Coniglio and Clemens, 1976) can produce irreversible alterations of reproductive morphology and behavior in males and females respectively.

Female hamsters exposed to 100 micrograms of estrogen during this period were defeminized and masculinized by treatment. Seventy-five percent of these females had abnormal behavioral estrous cycles being receptive more often than once every four days, and 73% mounted a receptive female as compared to 0% of controls in Experiment 2. It is clear that the absence of estrous cyclicity as a con-

sequence of estradiol treatment dose not imply an inability to display female sexual behavior. The lower dose of Estrogen (20 micrograms) and the 500 and 1000 microgram dosages of Chlordecone masculinized but did not defeminize the treated females. These females had normal behavioral estrous cycles but displayed abnormal sexual behavior by mounting a receptive female. These results demonstrate that high doses (500 and 1000 micrograms) of Chlordecone produce effects similar to the lower dose (20 micrograms) of estradiol benzoate. These results would indicate that Chlordecone behaves as a weak estrogen in the brain of the neonatal female hamster. It is likely that Chlordecone, which binds to the estrogen receptor in the uterus and *in vitro*, also binds to the cytosolic receptor in the brain and subsequently migrates to the nucleus inducing those areas of the brain responsible for masculine sexual behavior to differentiate like the brain of a male hamster. Therefore, when these treated females are exposed to testosterone as adults they respond like a normal male and mount a receptive female, whereas the untreated female's brain cannot respond to the activational influence of the male sex hormone. The observation that masculinization is more easily achieved in the female hamster than is defeminization using estrogen or Chlordecone, is consistent with the results of Whitsett and Vandenbergh (1975) who reported that masculinization effects could be obtained at doses 2 orders of magnitude below those that result in defeminization.

This type of chemical alteration of sexually dimorphic behavioral development is possible following genetic sex determination at fertilization because there are a number of critical developmental steps during which the mammalian embryo differentiates from an indifferent state with bisexual potential into a male or female (Naftolin, 1981). In the first step of this process H-Y antigen in the male induces the different gonad to develop as a male organ, while the absence of this organizing factor results in the differentiation of an ovary (Gordon and Ruddle, 1981; Haseltine and Ohno, .981). Up to this point each fetus has separate anlagen for both the male and female reproductive tract. Subsequent to gonadal differentiation the fetal testis produces testosterone and other factors which cause the male tract to develop and the female tract to regress while the lack of these influences result in the formation of a female tract and regression of the male tract (Wilson *et al.*, 1981).

Similar to the described anatomical bisexual potential, the mammalian fetus also possesses bisexual behavior potential and androgen present during a critical period induces male behavioral and physiological traits, an effect which can be chemically induced independent of the genetic sex (Harris and Levine, 1965; Young *et al.*, 1964; MacLusky and Naftolin, 1981). Testosterone directs the differentiation of the brain from its indifferent state into a male brain whereas in the female the lack of testicular hormones causes the brain to differentiate as a female brain. During sex differentiation of the brain testosterone is metabolized by neural tissue to estradiol which induces the differentiation process of some sexually dimorphic behaviors. For this reason exogenous estrogens and the weakly estrogenic pesticide Chlordecone masculinize behavioral sex differentiation as well as androgens. The brain of the normal male is both masculinized and defeminized to varing degrees, depending upon the species (Baum, 1979; Fig. 1, after Whitsett and Vandenbergh, 1978). Numerous studies have shown that exposing the female to estrogen masculinizes the brain with respect to most of the sexually dimorphic behaviors, and the brain is then able to respond to the activational influence of testosteone treat-

ment of female as an adult (Whitsett *et al.*, 1978). This activation occurs normally at puberty in the male, and these females display male sexual behavior under the appropriate testing conditions. Results of the present study clearly demonstrate that exposure to either estradiol benzoate of the weakly estrogenic pesticide Chlordecone during the sensitive period of sexual differentiation masculinizes the brain of the female hamster (Fig. 1).

While mounting behavior in the hamster is a sexually dimorphic behavior that requires both the organizational and activational influences of the gonadal hormones, other sexually dimorphic behaviors like the open field behaviors measured in the present study, are influenced by the organizational effects of sex hormones but do not require activation. Both intact and ovariectomized female hamsters ambulate more than males, demonstrating that estradiol does not activate open field ambulation; females masculinized as neonates with high doses of estradiol (150 micrograms/pup) (Swanson, 1966) ambulate less, thus demonstrating the organizational effect of estradiol. Other sexually dimorphic behaviors, such as wheel-running in the rat (Beatty, 1979), are primarily influenced by the activational influence of the sex hormones after puberty. Those sexually dimorphic behaviors in which organizational influences predominate are likely to be affected by Chlordecone as neonate whereas those regulated primarily by activatinal influences will not. Results of the open field tests in the present study demonstrate that this type of sexually dimorphic behavior, one which is influenced primarily by organizational effects of gonadal hormones, is also masculinized by Chlordecone treatment on day 4 and indicates that an alteration of behavioral sex differentiation affects many behaviors in addition to those associated with successful reproduction.

It is important to note that, even among rodents, there are large species differences in the hormonal determination of the noncopulatory behaviors. For example, running wheel activity is sexually dimorphic in the rat being strongly activated by estradiol, but not in the hamster where both males and females run extensively in the wheels, with the male running slightly more than the female (Gray *et al.*, 1981). Play behavior is sexually dimorphic in many species including rats (Meany and Stewart, 1981), hamsters (Goldman and Swanson, 1974), primates (Goy and Goldfoot, 1974) and man (Blurton-Jones, 1976). In all the aforementioned species males engage in more rough and tumble or agresssive play than do females. This behavior is influenced by the organizational affects of the sex hormones during differentiation. Injections of testosterone to rats (Meany and Stewart, 1981) and primates (Goy and Goldfoot, 1974) organize the brain in the male direction, and the exposed females engage in more agressive play than do normal females. In humans, a similar clinical situation exists in females suffering from the adrenogenital syndrome, which is a genetic disorder which results in excessive release of adrenal androgens. Presumably, as a result of prenatal exposure to androgen, these females engage in more aggressive play than normal girls do (Ehrhardt and Barker, 1974); Money and Ehrhardt, 1973; Ehrhardt and Meyer-Bahlburs, 1981). Although play behavior might have been measured in the present study, recent evidence suggests that this behavior differs from other sexually dimorphic behaviors in which organizational influences predominate, in that neonatal estradiol is without effect. Testosterone and dihydrotestosterone (an androgen that cannot be metabolized to estradiol), however, effectively organize the brain with respect to the play behaviors (Meany and Stewart, 1981). These results suggest that the hormone respon-

sible for behavioral sex differentiation differs from one sexually dimorphic behavior to another, and that testosterone is a prohormone that is metabolized to both estradiol, which masculinizes copulatory behavior and open field activity, and DHT, which organizes play behavior. Therefore, we would expect neonatal exposure to Chlordecone to masculinize those behaviors which are organized by estradiol, like mounting and open field behaviors, but not play behavior for which estradiol is ineffective.

The present study examined the effects of chlordecone administration during a single sensitive developmental period. Although behavioral sex differentiation occurs shortly after birth in rodents with a short gestation like mice, rats and hamsters, this critical developmental event occurs prenatally in humans. Therefore, the injection protocol used in the present study is equivalent to prenatal exposure in humans. It is important to note that there are many other critical or sensitive stages of reproductive and behavioral development that extended much later into postnatal life and exposure to hormonally active toxic chemicals during these periods may also produce severe and irreversible effects. We are currently investigating the ability of toxic chemicals to alter the onset of puberty and its associated behavioral changes.

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